

Serial No. 10/611,748
Amendment Dated March 20, 2007
Reply to Office Action of September 27, 2006

Amendments to the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

1. (Currently Amended) A gene silencing site-specific recombination system comprising:
 - a) a first recombinase element having the general structure P1-R, wherein P1 is a first promoter and R is a recombinase coding sequence and 3' region; and
 - b) a second gene silencing-recombinase element having the general structure: RS-X-RS*-Y
wherein:
 - i) RS and RS* are opposingly oriented recombinase sites responsive to the recombinase;
 - ii) X is a nucleic acid fragment comprising at least one second promoter in a 3' to 5' orientation, wherein X comprises 5' Intron-TSINV-P2INV; and
 - iii) Y is a nucleic acid fragment comprising at least one target sequence directed to a target gene, wherein Y comprises 3' Intron-TSINV-polyA;
wherein:
 - 1) P2INV comprises an inverted second promoter whose orientation is from 3'-5';
 - 2) TSINV is an inverted target sequence whose orientation is from 3'-5';

- 3) polyA is the 3' region of a gene;
- 4) 5' Intron is the N-terminal portion of an intron; and,
- 5) 3' Intron is the C-terminal portion of an intron;

wherein expression of the recombinase results in inversion of the element contained between RS and RS* and transcription of the inverted second gene silencing-recombinase element resulting in the production of double-stranded RNA and silencing of the target gene.

2. (Currently Amended) A The gene silencing site-specific recombination system according to claim 1 wherein the target sequence of Y is in a 3' to 5' orientation.
3. (Withdrawn-Currently Amended) A The gene silencing site-specific recombination system according to claim 1 wherein X is selected from the group consisting of: P2INV, TSINV-P2INV, and 5' Intron-TSINV-P2INV, and wherein Y is selected from the group consisting of TS-P3INV, TSINV-polyA, 3' Intron-TSINV-polyA, wherein:
 - a) P2INV and P3INV are inverted second and third promoters whose orientation is from 3'-5';
 - b) TS is a target sequence;
 - c) TSINV is an inverted target sequence whose orientation is from 3'-5';
 - d) polyA is the 3' region of a gene;
 - e) 5' Intron is the N-terminal portion of an intron; and
 - f) 3' Intron is the C-terminal portion of an intron.
4. (Cancelled)

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5. (Currently Amended) A The gene silencing site-specific recombination system according to any of Claims Claim 1 or 4 wherein the recombinase and recombinase site are selected from the group consisting of Cre-lox, FLP/FRT, R/RS, Gin/gix, a pSR1 system, a cer system, and a fim system.
6. (Currently Amended) A The gene silencing site-specific recombination system according to any of Claims Claim 1 or 4 wherein the first promoter and at least one second promoter are selected from the group consisting of
 - a) constitutive plant promoters;
 - b) plant tissue-specific promoters;
 - c) plant development stage-specific promoters;
 - d) chemically-inducible plant promoters; and
 - e) viral promoters.
7. (Withdrawn-Currently Amended) A gene silencing site-specific recombination system comprising:
 - a) a first recombinase element having the general structure P1-R, wherein P1 is a first promoter and R is a recombinase coding sequence and 3' region; and
 - b) a second ~~gene silencing~~-recombinase element having the general structure: RS-TSINV- P2INV-RS*- TSINV -polyA, wherein:
 - i) RS and RS* are opposingly oriented recombinase sites responsive to the recombinase;
 - ii) TSINV is an inverted target sequence whose orientation is from 3'-5', wherein the target sequence is directed to a target gene;
 - iii) P2INV is an inverted second promoter whose orientation is from 3'-5'; and
 - iv) polyA is the 3' region of a gene;

wherein P1 and P2 are operably linked to their down stream elements and wherein expression of the recombinase results in inversion of the element contained between RS and RS* and transcription of the ~~gene-silencing~~-recombinase element resulting in production of double-stranded RNA and silencing of the target gene.

8. (Currently Amended) A gene silencing site-specific recombination system comprising:
 - a) a first recombinase element having the general structure P1-R, wherein P1 is a first promoter and R is a recombinase coding sequence and 3' region; and
 - b) a second ~~gene-silencing~~-recombinase element having the general structure RS-5' Intron-TSINV- P2INV-RS*-3' Intron-TSINV -polyA, wherein:
 - i) RS and RS* are opposingly oriented recombinase sites responsive to the recombinase;
 - ii) 5' Intron is the N-terminal portion of an intron;
 - iii) TSINV is an inverted target sequence and whose orientation is from 3'-5', wherein the target sequence is directed to a target gene;
 - iv) P2INV is an inverted second promoter whose orientation is from 3'-5';
 - v) 3' Intron is the C-terminal portion of an intron; and
 - vi) polyA is the 3' region of a gene;wherein P1 and P2 are operably linked to their down stream elements and wherein expression of the recombinase results in inversion of the element contained between RS and RS* and transcription of the inverted second gene silencing-recombinase element, resulting in excision of the intron by mRNA splicing and production of double-stranded RNA and silencing of the target gene.

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9. (Withdrawn-Currently Amended) A gene silencing site-specific recombination system comprising:
 - a) A recombinase element having the general structure P1-R, wherein P1 is a first promoter and R is a recombinase coding sequence and 3' region; and
 - b) A ~~gene silencing~~-recombinase element having the general structure RS-P2INV-RS*-TS-P3 INV, wherein:
 - i) RS and RS* are opposingly oriented recombinase sites responsive to the recombinase;
 - ii) P2INV and P3 INV are inverted second and third promoters respectively, whose orientation is from 3'-5'; and
 - iii) TS is a target sequence;wherein P1, P2, and P3 are operably linked to their down stream elements and wherein expression of the recombinase results in inversion of the element contained between RS and RS* and transcription of the ~~gene silencing~~-recombinase element resulting in production of double-stranded RNA silencing the target gene.
10. (Withdrawn-Currently Amended) A The gene silencing site-specific recombination system according to Claim 9 wherein the target sequence has a poly A region operably-linked at its 3' end.
- 11-12 (Cancelled)
13. (Currently Amended) A The gene silencing site-specific recombination system according to any one of Claims ~~8-12~~ 8-10 wherein the first promoter is a germline promoter.

14. (Currently Amended) A The gene silencing site-specific recombination system according to Claim 14 13 wherein the germline promoter is selected from the group consisting of:
 - a) constitutive plant promoters;
 - b) plant tissue-specific promoters;
 - c) plant developmental stage-specific promoters;
 - d) chemically-inducible plant promoters;
 - e) viral promoters;
 - f) male germline-specific promoters;
 - g) female germline-specific promoters;
 - h) common germline-specific promoters;
 - i) floral common germline-specific promoters;
 - j) vegetative shoot apical meristem-specific promoters; and
 - k) floral shoot apical meristem-specific promoters.
15. (Currently Amended) A The gene silencing site-specific recombination system according to Claim 14 wherein the male germline-specific promoter is derived from genes selected from the group consisting of genes specific to anther primordia, anther sporophyte and pollen gametophyte.
16. (Currently Amended) A The gene silencing site-specific recombination system according to Claim 14 wherein the common germline-specific promoter is derived from genes selected from the group consisting of Apetala 3 (AP3), Pistillata (PI), synthetic anther promoter, TA29, BCP1 and orthologs thereof.
17. (Currently Amended) A The gene silencing site-specific recombination system according to Claim 16 wherein the common germline-specific promoter is

derived from genes selected from the group consisting vegetative and floral shoot apical meristems.

18. (Currently Amended) A The gene silencing site-specific recombination system according to Claim 48 17 wherein the common germline-specific promoter is derived from genes selected from the group consisting of Leafy (LFY), Apetala 3 (AP3), Pistillata (PI), Apetala 1 (AP1), Agamous (AG), Pistillata (PI) and orthologs thereof.

19. (Currently Amended) A The gene silencing site-specific recombination system according to Claim 14 wherein the floral common germline-specific promoter is derived from genes selected from the group consisting of Agamous (AG), Apetala 1 (AP1), Apetala 3 (AP3), Leafy (LFY) and orthologs thereof.

20. (Currently Amended) A The gene silencing site-specific recombination system according to Claim 14 wherein the vegetative shoot apical meristem-specific promoter is selected from the group consisting of Agamous (AG), Apetala 1 (AP1), Apetala 3 (AP3), Leafy (LFY), Aintegumenta (ANT), Clavata 3 (CLV3), Wushel (WUS), Meristemless (STM) and orthologs thereof.

21. (Currently Amended) A The gene silencing site-specific recombination system according to any of Claims 7-9 and 11-12 wherein the second promoter and optionally, third promoter, are selected from the group consisting of:

- constitutive plant promoters;
- plant tissue-specific promoters;
- plant development stage-specific promoters;
- chemically-inducible plant promoters; and
- viral promoters.

22. (Currently Amended) A The gene silencing site-specific recombination system according to any of Claims 1, 4 and 7-9 and ~~11-12~~ wherein the target sequence silences a target gene selected from the group consisting of:

- a) a gene encoding an enzyme of a biosynthetic pathway;
- b) a gene encoding a storage protein;
- c) a gene conveying sterility;
- d) a gene conveying a specific phenotype on a plant or plant cell;
- e) a hormone biosynthetic gene; and
- f) a gene involved in gene silencing.

23. (Currently Amended) A The gene silencing site-specific recombination system according to Claim 22 wherein the genes involved in gene silencing are selected from the group consisting of:

- a) qde-1, qde-2, and qde-3;
- b) rde-1, rde-2, rde-3, and rde-4;
- c) mut-2, and mut-7;
- d) ego-1;
- e) AGO1;
- f) SGS-2/SDE-1, SGS-1, and SGS-3;
- g) RdRP; and,
- h) Dicer; and
- i) ~~homologs of thereof.~~

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24. (Currently Amended) A The gene silencing site-specific recombination system according to any of Claims 7-9 and ~~11-12~~ wherein the recombinase coding sequences and recombinase site are selected from the group consisting of Cre-lox, FLP/FRT, R/RS, Gin/gix, a pSR1 system, a cer system, and a fim system.
25. (Currently Amended) A The gene silencing site-specific recombination system according to any of Claims 7-9 and ~~11-12~~ wherein the first recombinase element and the second gene silencing-recombinase element may be genetically linked or unlinked.
26. (Currently Amended) A The gene silencing site-specific recombination system according to Claim 25 wherein the first recombinase element and the second gene silencing-recombinase element may be are genetically unlinked and reside in different plants.

27-38 (Cancelled)